Research Article

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COMPARATIVE ANTIMICROBIAL STUDY OF DIFFERENT EXTRACTS OF *BARLERIA LUPULINA* LINDL. AS A POTENTIAL SUBSTITUTE OF *BARLERIA PRIONITIS* LINN. (KATSAIREYA) IN INFECTIOUS DISEASES

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ABSTRACT

Katsaireya (*Barleria prionitis* Linn.) is mentioned in Ayurvedic classics for Krimighana (Antimicrobial), Vranaropana (wound healing), and Shothahara (Anti-inflammatory) indicating its antimicrobial, anti-inflammatory and wound healing properties. Another extrapharmacoepial Barleria species – *Barleria lupulina* Lindl. having similar phytochemical properties is compared with *Barleria prionitis* for antimicrobial action in the present study. Aqueous, ethanolic, and methanolic extracts of both the samples were tested against *Escherichia coli* (Gram Negative) and *Staphylococcus aureus* (Gram-Positive) bacteria using Kirby–Bauer Disc Diffusion and Agar Well Diffusion methods. *Barleria lupulina* exhibited greater antimicrobial activity than *Barleria prionitis*, with the aqueous extract showing the highest inhibition zone (22 mm) against *Staphylococcus aureus* and the methanolic extract significantly inhibited *Escherichia coli*. Antimicrobial activity increased with higher concentrations, and the Agar Well Diffusion method was found to be more effective than the Disc Diffusion method. The study concludes that *Barleria lupulina* possesses stronger antibacterial potential and may serve as an effective substitute for *Barleria prionitis* in Ayurvedic formulations targeting microbial infections.

Keywords: *Barleria prionitis*, *Barleria lupulina*, *Escherichia coli*, *Staphylococcus aureus*, antimicrobial activity, Krimighana, Vranaropana

INTRODUCTION

Microorganisms play a crucial role in maintaining ecological balance; however, they are responsible for a wide range of infectious diseases also. The indiscriminate and prolonged use of antibiotics has resulted in the emergence of antimicrobial resistance, creating an urgent need to explore alternative and safer antimicrobial agents from natural sources.[1] Medicinal plants are rich reservoirs of bioactive phytochemicals such as alkaloids, flavonoids, tannins, phenolics and glycosides, which contribute to their antimicrobial, anti-inflammatory and wound-healing properties.[2,3]

The genus *Barleria* (family Acanthaceae) is widely recognized in Ayurveda for its extensive therapeutic potential and is distributed throughout tropical and subtropical regions.[4] *Barleria prionitis* Linn. (Katsaireya) is of repute by the name of Vajradanti, used for dental hygiene and described in classical Ayurvedic texts as Vatashamaka, Krimighna (Anti-microbial), Vranashodhana (Wound cleamsing), Vranaropana (Wound healing), Shothahara (Anti-inflammatory), Vishaghna (Antitoxin) and Kandughna (Anti-pruritic).[5,6,7] Traditionally, it is used in the management of wounds, boils, eczema, inflammatory conditions, oral disorders and neurological ailments.[8] Pharmacological studies have demonstrated that various parts of *B. prionitis*, particularly leaves and roots, possess significant antimicrobial, antioxidant, anti-inflammatory, hepatoprotective and analgesic activities.[9,10]

These effects are attributed to the presence of iridoid glycosides such as barlerin, acetylbarlerin, shanzhiside methyl ester and 6-O-acetyl shanzhiside methyl ester, along with flavonoids and phenolic compounds. The antimicrobial activity of *B. prionitis* has been linked to phytoconstituents including acetylbarlerin, barlerin, verbascoside, balarenone, pipataline and steroidal compounds such as 13,14-seco-stigmasta-5,14-diene-3-α-ol.[11,12]

Similarly, *Barleria lupulina* Lindl. (Hophead or Philippine violet) is an ethnomedicinal plant traditionally employed in the treatment of snake and insect bites, skin infections, abscesses, herpes and inflammatory conditions but is not documented in Ayurveda literature which makes it Anukta/extrapharmacopoieal drug.[13] Phytochemical investigations of *B. lupulina* leaves have also revealed the presence of iridoid glucosides such as barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester, ipolamiidoside and other iridoid glycosides.[10] Extracts from its leaves and flowers contain potent bioactive compounds including verbascoside, barlerinoside, lupulin A and B, and apigenin derivatives, which contribute to its antimicrobial and antioxidant activities.[14]

The pharmacological similarities between *B. prionitis* and *B. lupulina*, along with their documented antimicrobial potential and classical Ayurvedic relevance, provide a strong scientific rationale for the comparative evaluation of these plants against *S. aureus* and *E. coli* using standard in vitro antimicrobial methods.

MATERIALS AND METHODS

Collection of Plant Materials: Fresh whole plants of *B. prionitis* (Sample A) and *B. lupulina* (Sample B) were collected from the Herbal Garden, Government Ayurvedic College, Patiala, Punjab, India. The taxonomic identities of both plants were confirmed by the subject experts in the PG Department of Dravyaguna, Government Ayurvedic College, Patiala, Punjab, India.

Experimentation and Observation: A comparative antimicrobial study against *Escherichia coli* (Gram Negative) and *Staphylococcus aureus* (Gram-Positive) bacteria was designed using Kirby–Bauer Disc Diffusion and Agar Well Diffusion methods.

Preparation of Extracts: Aqueous, ethanol, and methanol extracts of both the samples were prepared using the Soxhlet apparatus at the Herbal Health Research Consortium, Amritsar. DMSO was used as solvent control and diluent.

Dilution for Kirby–Bauer Disc Diffusion Method: Pure extracts (100%) were used in graded doses of 10 µl, 20 µl, and 30 µl per disc.

Dilution for Agar Well Diffusion Method: Extracts were diluted with DMSO to obtain 50%, 80%, and 100% concentrations.

Test Microorganisms: Standard strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were procured from the Department of Microbiology, Government Medical College, Patiala, and revived using appropriate nutrient media. The strains were subcultured on MacConkey Agar (for *E. coli*) and Blood Agar (for *S. aureus*) and incubated at 37°C for 24 hours. Gram staining and biochemical tests confirmed their identity.

Confirmatory Tests

The bacterial isolates, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), were subjected to standard biochemical confirmatory tests to verify their identity and purity prior to antimicrobial evaluation. *E. coli* appeared as Gram-negative, rod-shaped bacteria forming pink colonies on MacConkey agar, indicating lactose fermentation. Biochemical confirmation included a positive Indole test (Figure 1), where a red ring developed after adding Kovac's reagent, confirming indole production from tryptophan, and a negative Urease test (Figure 2), in which the medium remained yellow, indicating the absence of urease enzyme. *S. aureus* colonies appeared golden-yellow on Blood agar and were Gram-positive cocci arranged in clusters. Their identity was further confirmed by a positive Catalase test, showing effervescence upon addition of hydrogen peroxide due to catalase enzyme activity, and a positive Tube Coagulase test, which demonstrated clot formation after incubation, confirming the presence of free coagulase enzyme. These confirmatory tests collectively validated the authenticity and purity of both bacterial strains used for subsequent antimicrobial assays.

Figure 1: Indole Test

Figure 2: Urease Test

Antimicrobial Susceptibility Testing

Kirby–Bauer Disc Diffusion Method: Sterile Mueller–Hinton Agar (MHA) plates were prepared, and bacterial suspensions standardized to 0.5 McFarland (≈1.5×10^8 CFU/mL) were swabbed uniformly over the surface. Sterile Whatman No. 3 paper discs (6 mm diameter) were mpregnated with 10 µl, 20 µl, and 30 µl of each extract (aqueous, ethanol, and methanol) and air-dried under aseptic conditions. These discs were then carefully placed on the inoculated agar surface using sterile forceps, along with standard antibiotic discs (Ab)—Nitrofurantoin and Norfloxacin for E. coli, and Amoxycillin and Gentamicin for S. aureus—serving as positive controls. Plates were incubated at 37°C for 18–24 hours, and the diameter of the Zone of Inhibition (ZOI) was measured in millimeters.

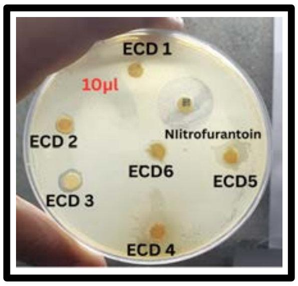


Figure 3: ZOI of different pure extracts at 10µl dose of each of both the samples and Antibiotic against *E. coli* by Kirby–Bauer Disc Diffusion Method

Figure 4: ZOI of different pure extracts at 10µl dose of each of both the samples and Antibiotic against *Staphylococcus aureus* by Kirby–Bauer Disc Diffusion Method.

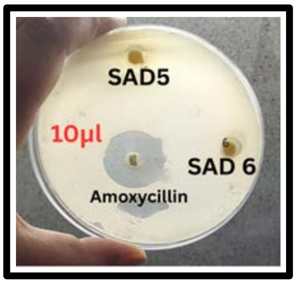


Figure 5: ZOI of different pure extracts at 10µl dose of each of both the samples and Antibiotic against *Staphylococcus aureus* by Kirby–Bauer Disc Diffusion Method

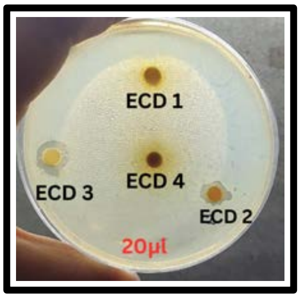


Figure 6: ZOI of different pure extracts at 20µl dose of each of both the samples and Antibiotic against *E. coli* by Disc Diffusion Method

Figure 7: ZOI of different pure extracts at 20µl dose of each of both the samples and Antibiotic against *E. coli* by Disc Diffusion Method

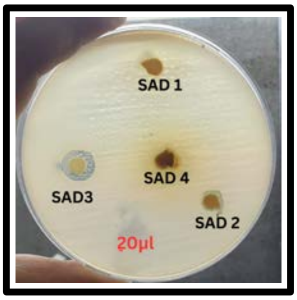


Figure 8: ZOI of different pure extracts at 20µl dose of each of both the samples and Antibiotic against *Staphylococcus aureus* by Disc Diffusion Method

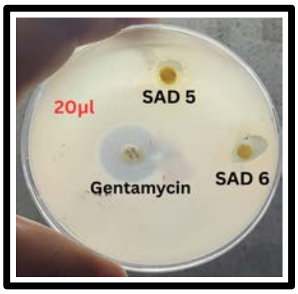


Figure 9: ZOI of different pure extracts at 20µl dose of each of both the samples and Antibiotic against *Staphylococcus aureus* by Disc Diffusion Method

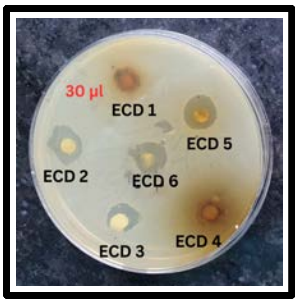


Figure 10: ZOI of different pure extracts at 30µl dose of each of both the sample against *E. coli* (Figure 7) *and Staphylococcus aureus* (Figure 8) by Disc Diffusion Method

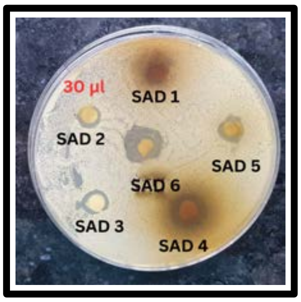


Figure 11: ZOI of different pure extracts at 30µl dose of each of both the sample against *E. coli* (Figure 7) *and Staphylococcus aureus* (Figure 8) by Disc Diffusion Method

*Here EC refers to *E. coli*, SA refers to *Staphylococcus aureus*, D refers to Disc diffusion method and 1, 2, 3 etc. refers to different extracts of *B. prionitis* and 4,5 and 6 refers to different extracts of *B. lupulina*

RESULTS

Table 1: Antimicrobial activity of different pure extracts at 10 µl, 20 µl, 30 µl dose of each of both the samples against *Escherichia coli* by Disc Diffusion Method. (Figures 3,6,7 and 10)

	ZOI (mm)							
	Barleria prionitis				***Barleria lupulina***			
Extract	**Name**	**10µl**	**20µl**	**30µl**	**Name**	**10µl**	**20µl**	**30µl**
Aqueous	ECD 1.	06	06	06	ECD 4.	06	06	06
Ethanol	ECD 2.	07	10	13	ECD 5.	12	13	13
Methanol	ECD 3.	08	10	13	ECD 6.	08	10	12

* Here EC refers to *E. coli*, SA refers to *Staphylococcus aureus*, D refers to Disc diffusion method and 1, 2, 3 etc. refers to different extracts of *B. prionitis* and 4,5 and 6 refers to different extracts of *B. lupulina.*

Table 2: Antimicrobial activity of standard Antimicrobial Susceptibility discs: Nitrofurantoin & Norfloxacin against *Escherichia coli* (Figures 3 and 7)

S. No.	Antimicrobial Susceptibility disc	ZOI (mm)	Sensitivity
ECD 7.	Nitrofurantoin	21	Sensitive
ECD 8.	Norfloxacin	27	Sensitive

*CLSI guidelines 2023 33rd edition

**Here EC refers to *Escherichia coli*, D refers to disc diffusion method and 7, 8 refers to Antibiotics as positive control.

Table 3: Antimicrobial activity of different pure extracts at 10 µl, 20 µl, 30 µl dose of each of both the samples against *Staphylococcus aureus* by Kirby–Bauer Disc Diffusion Method. (Figures 4,8,9 and 11)

	ZOI (mm)							
	Barleria prionitis				***Barleria lupulina***			
Extract	**Name**	**10µl**	**20µl**	**30µl**	**Name**	**10µl**	**20µl**	**30µl**
Aqueous	SAD 1.	06	06	06	SAD 4.	06	08	13
Ethanol	SAD 2.	08	08	08	SAD 5.	09	10	10
Methanol	SAD 3.	09	10	10	SAD 6.	09	13	13

*Here SA refers to *Staphylococcus aureus*, D refers to disc diffusion method and 1, 2, 3 etc. refers to different extracts of *B. prionitis* and 4,5 and 6 refers to different extracts of *B. lupulina.*

Table 4: Antimicrobial activity of standard Antimicrobial Susceptibility discs: Amoxycillin & Gentamicin against *Staphylococcus aureus*: (Figures 5 and 9)

S. No.	Antimicrobial Susceptibility disc	ZOI (mm)	Sensitivity
SAD 7.	Amoxycillin	23	Sensitive
SAD 8.	Gentamicin	21	Sensitive

*CLSI guidelines 2023 33rd edition

**Here SA refers to *Staphylococcus aureus*, D refers to disc diffusion method and 7, 8 refers to Antibiotics as positive control.

Agar Well Diffusion Method

MHA plates were inoculated again with standardized bacterial suspensions, and wells (6 mm in diameter) were made using a sterile cork borer. The extracts were diluted with Dimethyl Sulfoxide (DMSO) to obtain concentrations of 50%, 80%, and 100 %. Each well was filled with 100 µL of the respective dilution using a micropipette. DMSO served as the negative control, while ethanol and methanol were used as neutral controls. The plates were incubated at 37°C for 18–24 hours, and the zones of inhibition (ZOIs) were measured in millimeters. Solvents (Ethanol and Methanol) showed no inhibition, confirming the antimicrobial potential of the plant extracts only.

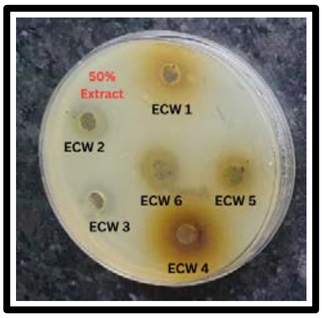


Figure 12: ZOI at 50% Extract Concentration

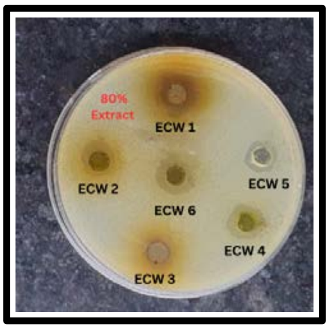


Figure 13: ZOI at 80% Extract Concentration

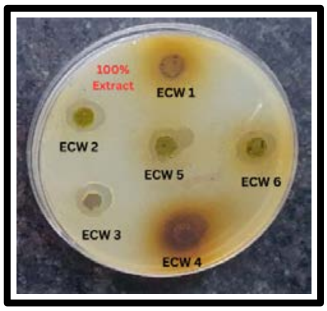


Figure 14: ZOI at 100% Extract Concentration. ZOI of three different extracts of both the samples each at 100µl dose of 50%, 80% & 100% extract conc. diluted with DMSO against *Escherichia coli* by Agar Well Diffusion Asay Method.

Figure 15: ZOI at 50% Extract Concentration

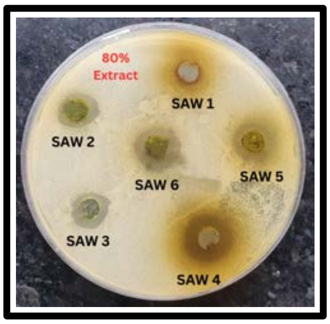


Figure 16: ZOI at 80% Extract Concentration

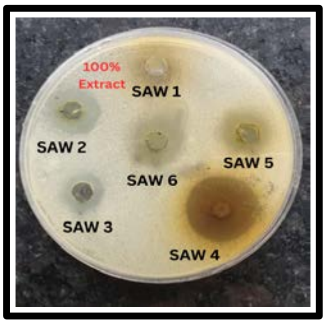


Figure 17: ZOI at 100% Extract Concentration

Table 5: Antimicrobial activity of three different extracts of both the samples each at 100µl dose of 50%, 80% & 100% extract conc. diluted with DMSO against *Escherichia coli* by Agar Well Diffusion Asay Method. (Figures 12-14)

	ZOI (mm)							
	Barleria prionitis				*Barleria lupulina*			
Extract	Name	10µl	20µl	30µl	Name	10µl	20µl	30µl
Aqueous	ECW 1.	06	06	06	ECW 4.	11	13	13
Ethanol	ECW 2.	12	12	14	ECW 5.	12	12	14
Methanol	ECW 3.	11	12	14	ECW 6.	12	14	15

*Here EC refers to *E. coli*, W refers to Well diffusion method and 1, 2, 3 etc. refers to different extracts of *B. prionitis* and 4,5 and 6 refers to different extracts of *B. lupulina*.

Table 6: Antimicrobial activity of pure solvents (Ethanol and Methanol) at 100µl dose

S. No.	Extract	Zone of inhibition (mm)
ECW 7.	Ethanol	6
ECW 8	Methanol	6

**Here EC refers to *E. coli*, W refers to Well diffusion method and 7, 8 refers to ethanol & methanol were used as neutral control

Table 7: Antimicrobial activity of three different extracts of each at 100µl dose of 50%, 80% & 100% Extract conc. diluted with DMSO against *Staphylococcus aureus* by Agar Well Diffusion Assay Method. (Figures 15-17)

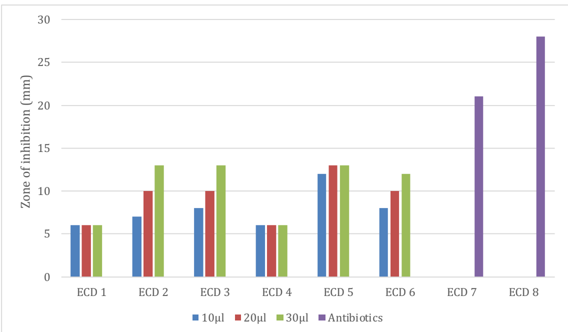
	ZOI (mm)							
	Barleria prionitis				***Barleria lupulina***			
Extract	**Name**	**10µl**	**20µl**	**30µl**	**Name**	**10µl**	**20µl**	**30µl**
Aqueous	SAW 1.	06	06	07	SAW 4.	13	14	22
Ethanol	SAW 2.	06	11	13	SAW 5.	13	13	16
Methanol	SAW 3.	10	11	11	SAW 6.	13	13	15

**Here SA refers to *Staphylococcus aureus*, W refers to Well diffusion method and 1, 2, 3 etc. refers to different extracts of *B. prionitis* and 4,5 and 6 refers to different extracts of *B. lupulina.*

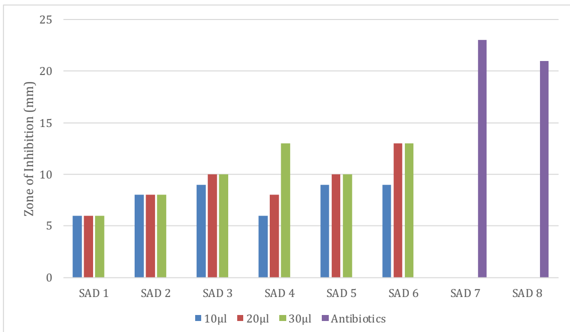
Table 8: Antimicrobial activity of Pure solvents (Ethanol and Methanol) at 100µl

S. No.	Extract	Zone of inhibition (mm)
SAW 7.	Ethanol	6
SAW 8.	Methanol	6

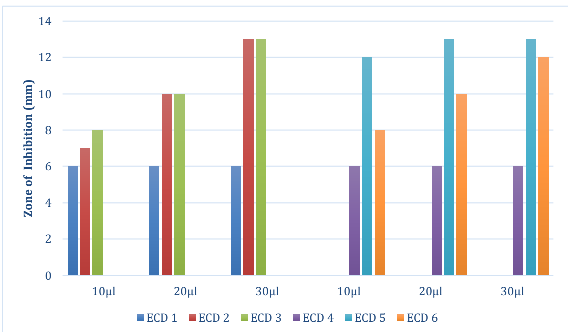
In both antimicrobial testing methods, *Barleria lupulina* exhibited higher antibacterial activity than *Barleria prionitis*. In the Kirby–Bauer disc diffusion method, methanolic and ethanolic extracts of *B. lupulina* produced zones of inhibition ranging from 12–13 mm against both *Escherichia coli* and *Staphylococcus aureus*, whereas *B. prionitis* showed comparatively lower inhibition zones of 8–10 mm. The aqueous extracts of both plant samples demonstrated minimal inhibitory activity in this method. Standard antibiotics—Nitrofurantoin (21 mm), Norfloxacin (27 mm), Amoxycillin (23 mm), and Gentamicin (21 mm)—showed greater inhibition In the agar well diffusion assay, the aqueous extract of *B. lupulina* showed the highest antibacterial activity with a zone of inhibition of 22 mm against *S. aureus*, while the methanolic extract exhibited the maximum inhibition of 15 mm against *E. coli*. The solvents used as controls (ethanol and methanol) did not show any inhibitory effect, confirming that the observed antimicrobial activity was due to the plant extracts only. Additionally, the antimicrobial effect increased with increasing extract concentration, and overall, the agar well diffusion method produced larger zones of inhibition compared to the disc diffusion method.



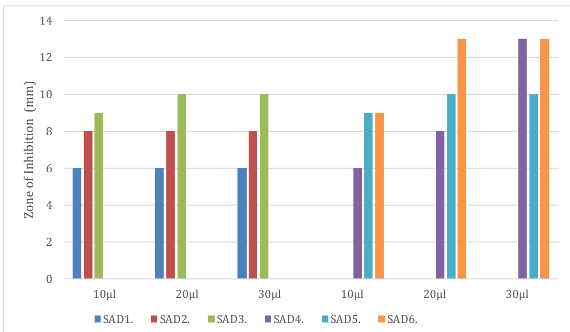
Graph 1: Antimicrobial activity of three different pure extracts at 10 µl, 20 µl, 30 µl dose of each of both the samples against *Escherichia coli* by Disc Diffusion Method. (Figures 3, 6, 7 and 10)



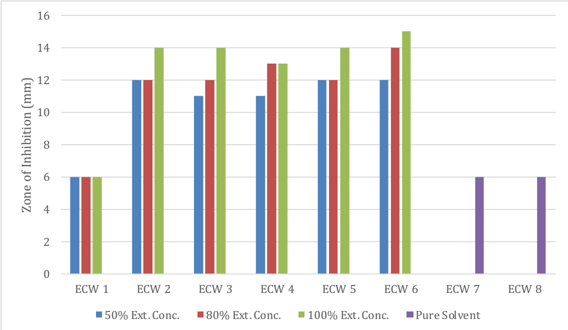
Graph 2: Antimicrobial activity of three different pure extracts at 10 µl, 20 µl, 30 µl dose of each of both the samples against *Staphylococcus aureus* by Disc Diffusion Method
(Figures 4,5,8,9 and 11)



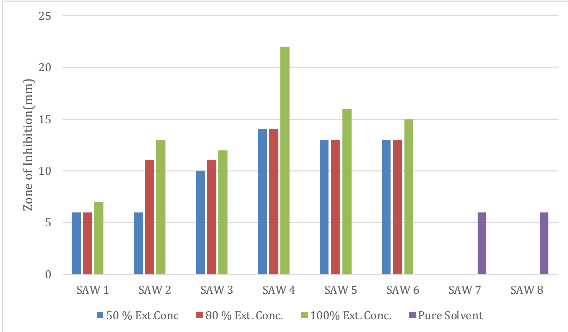
Graph 3: Antimicrobial activity of different pure extracts at 10 µl, 20 µl, 30 µl dose of both the samples against *Escherichia coli* by Disc Diffusion Method.



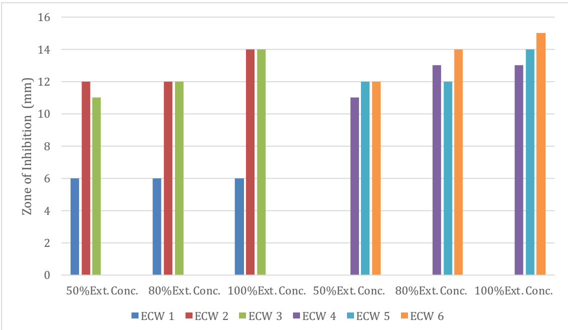
Graph 4: Antimicrobial activity of different pure extracts at 10 µl, 20 µl, 30 µl dose of both the samples against *Staphylococcus aureus* Disc Diffusion Method.



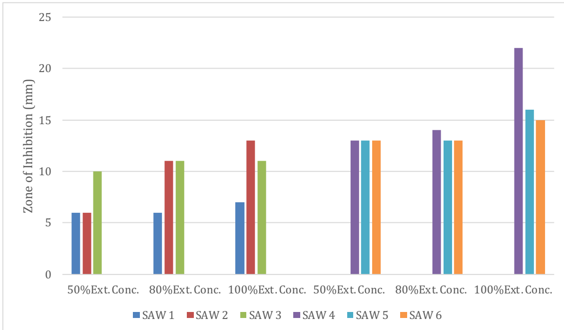
Graph 5: Antimicrobial activity of three different Pure extracts of both samples each at 100µl dose of 50%, 80% & 100% Ext. conc. diluted with DMSO *Escherichia Coli* microbe by Agar Well Diffusion Asay Method. (Figures 12-14)



Graph 6: Antimicrobial activity of three different Pure extracts of both samples each at 100µl dose of 50%, 80% & 100% Ext. conc. diluted with DMSO against *Staphylococcus aureus* microbe- by Agar Well Diffusion Asay Method. (Figures 15-17)



Graph 7: Antimicrobial activity of three different Pure extracts of both samples each at 100µl dose of 50%, 80% & 100% Ext. conc. diluted with DMSO against *Escherichia coli* by Agar Well Diffusion Asay Method.



Graph 8: Antimicrobial activity of three different Pure extracts of both samples each at 100µl dose of 50%, 80% & 100% Ext. conc. diluted with DMSO against *Staphylococcus aureus* by Agar Well Diffusion Asay Method.

*Here EC refers to *E. coli,* SA refers to *S. aureus*, D refers to Disc diffusion method, W refers to Well diffusion method, Whereas and 1, 2, 3 etc. refers to different extracts of *B. prionitis* and 4,5 and 6 refers to different extracts of *B. lupulina*.

DISCUSSION

The present comparative antimicrobial investigation of *Barleria prionitis* Linn. and *Barleria lupulina* Lindl. demonstrated that both plant species possess appreciable antibacterial activity against the tested organisms, namely *Staphylococcus aureus* and *Escherichia coli*. These findings support the traditional therapeutic claims of *B. prionitis* in Ayurveda as a Krimighna (antimicrobial) and Vranaropana (wound-healing) drug and also indicate the potential pharmacological value of *B. lupulina*, an extrapharmacopoeial species belonging to the same genus. However, the results clearly revealed that *B. lupulina* exhibited comparatively stronger and more consistent antibacterial activity than *B. prionitis* across different extracts and experimental methods.

The study also revealed that antimicrobial activity varied depending on the extraction solvent used. Methanolic and ethanolic extracts of both the drugs in general demonstrated higher antibacterial activity against *E.coli* compared to aqueous extracts in both the disc diffusion assay and Kirby Bauer methods, indicating that bioactive phytoconstituents extracted by organic solvents such as flavonoids, phenolics, and iridoid glycosides are better effective in controlling growth of *E.coli*. These compounds are known to possess strong antimicrobial properties through various mechanisms, including disruption of microbial cell membranes, inhibition of nucleic acid synthesis, interference with enzymatic activity, and induction of oxidative stress within microbial cells. In contrast, aqueous extracts may contain fewer non-polar or moderately polar phytochemicals, which may explain the comparatively lower activity against *E.coli.*

Interestingly, in the agar well diffusion assay, the aqueous extract of *B. lupulina* exhibited the highest inhibition zone (22 mm) against *S. aureus* and 13mm in Agar Well method. This result suggests that certain water-soluble phytochemicals present in *B. lupulina* may possess strong antibacterial properties against *S. aureus*. The enhanced activity in the well diffusion assay can also be attributed to the larger volume of extract applied (100 µL) and better diffusion of the sample through the agar medium compared with the impregnated discs used in the Kirby–Bauer method. Therefore, the agar well diffusion technique often produces wider zones of inhibition and is considered more sensitive when evaluating crude plant extracts.

The superior antimicrobial activity observed in *B. lupulina* may be explained by its phytochemical composition. Previous phytochemical investigations have identified the presence of several bioactive constituents in this species, including iridoid glucosides such as barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester and ipolamiidoside, along with phenylethanoid glycosides such as verbascoside and barlerinoside. These compounds are widely recognized for their antimicrobial, antioxidant, and anti-inflammatory activities. Flavonoids and phenolic compounds present in *Barleria* species are also known to destabilize microbial cell membranes and inhibit bacterial enzymes, ultimately leading to cell death. The higher concentration or better bioavailability of these phytoconstituents in *B. lupulina* could be responsible for its stronger antibacterial effect specially against *Staphylococus aureus* compared to *B. prionitis*.

From an Ayurvedic perspective, the results of this study correlate well with the traditional therapeutic indications of *B. prionitis*, which is described in classical texts as Krimighna (Antimicrobial), Vranashodhana (Antiseptic), Vranaropana (Wound healing), Kandughna (Antipruritic), and Shothahara (Anti-inflammatory activity). These pharmacological actions correspond to antimicrobial, wound-cleansing, wound-healing, antipruritic, and anti-inflammatory effects in modern biomedical terminology. Since *B. lupulina* belongs to the same botanical genus and exhibits similar phytochemical constituents, it may possess comparable pharmacodynamic properties. Although it is not mentioned in classical Ayurvedic texts, the strong antimicrobial activity observed in this study suggests that *B. lupulina* may be considered an Anukta or extrapharmacopoeial drug with therapeutic potential. Such plants are often explored as Pratinidhi Dravya (substitute drugs) when classical drugs are scarce or unavailable.

Another important observation in the present study was the dose-dependent increase in antimicrobial activity. As the concentration of extracts increased, the zones of inhibition also increased correspondingly. This pattern indicates a clear relationship between extract concentration and antibacterial efficacy, suggesting that the active phytochemicals exert a stronger inhibitory effect at higher concentrations. Such concentration-dependent activity is commonly observed in plant-based antimicrobial studies and supports the pharmacological validity of the tested extracts.

The use of standard antibiotic discs in the present study served as positive controls to validate the experimental methodology. The observed sensitivity of *E. coli* to Nitrofurantoin and Norfloxacin and of *S. aureus* to Amoxycillin and Gentamicin confirmed the reliability of the microbial cultures and assay conditions. Although the inhibition zones produced by plant extracts were comparatively smaller than those produced by standard antibiotics, the results are significant because crude plant extracts contain complex mixtures of compounds rather than purified active molecules. Further purification and isolation of active phytochemicals from *B. lupulina* may potentially lead to compounds with stronger antimicrobial activity.

Modern pharmacological studies have also reported various biological activities of *B. lupulina*, including antibacterial, antiviral, antidiabetic, antiulcer, antioxidant, and anti-inflammatory effects. The plant has demonstrated inhibitory activity against several pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Propionibacterium acnes*. In addition, virucidal activity against herpes simplex virus type-2 (HSV-2) and neuropharmacological effects have been reported in previous studies.[15] These findings support the observations of the present study and further highlight the broad therapeutic potential of *B. lupulina*.

Overall, the comparative evaluation indicates that while both *Barleria* species possess significant antimicrobial activity, *B. lupulina* demonstrates comparatively higher antibacterial potency. The presence of diverse phytoconstituents, particularly iridoid glycosides and phenolic compounds, may contribute to its enhanced antimicrobial effect. Therefore, *B. lupulina* may serve as a promising herbal alternative or substitute for *B. prionitis* in the management of microbial infections, particularly those associated with wound infections and skin disorders.

However, further studies involving minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), phytochemical isolation, and *in vivo* pharmacological evaluation are necessary to better understand the therapeutic potential and clinical applicability of this plant.

CONCLUSION

The present study concludes that both *Barleria prionitis* Linn. and *Barleria lupulina* Lindl. possess significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli,* supporting their traditional Ayurvedic uses as Krimighana (antimicrobial) and Vranaropana (wound healing) agents. Among the two, *B. lupulina* exhibited superior antibacterial efficacy, particularly in its aqueous and methanolic extracts, with the maximum inhibition zone (22 mm) observed against S. aureus. The enhanced activity may be attributed to the presence of bioactive compounds such as flavonoids, phenolics, and iridoid glycosides. The results suggest *that B. lupulina* can serve as a potential substitute for *B. prionitis* in Ayurvedic formulations and may be further explored as a promising source for the development of safe and effective herbal antimicrobial agents.

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